Places of Innovation, Sites of Discovery

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Premature and Postmature Scientific Discovery

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Although this workshop has been dedicated to "Places of Innovation, Sites of discovery", I wish to talk about "Premature and Postmature Scientific Discoveries."

My excuse is that, as the advertisement states [**Fig**]: "Albert Einstein figured out that time and space are relative," so time may be viewed as just another dimension of the space.

All of us are familiar with the notion of "premature" scientific discoveries. Coming from the discipline of Genetics, it is natural for me to mention Mendel's paper of 1864 (published in 1865) that was not appreciated until – some would say "was rediscovered in" – 1900. But one can similarly mention the rule of DNA as the genetic material.

Summing up their experimental effort that started in 1935, Oswald T.Avery, Colin MacLeod and Maclyn McCarty unequivocally showed in 1944 that the "transforming principle" of *Pneumococcus*, discovered in 1928 by Fred Griffith was 99% pure desoxyreibonucleic acid. It took, however, another eight years for the paper of Alfred D. Hershey and Martha Chase to convince the geneticists' community that DNA, rather than protein, was the genetic material. Notably, although the Hershey and Chase 1952 experiment was very elegant, it was much less clean than that of Avery et. al.: Not more than 80% of the marked DNA of the infecting phage entered the bacteria. The evidence was neither unknown nor mistrusted by geneticists – I myself was taught of Avery et al.'s experiment by Prof. Shaul Adler in 1950 – it was premature. Alfred Mirsky, of the Rockefeller Institute, insisted even in the beginning of the 1950s that the 1% or less protein contamination of the Avery et al.'s preparations, rather than the DNA, was responsible for the bacterial transformation.

Indeed, as noted by Gunther Stent, Geneticists "did not seem to be able to do much with it or build on it",

Thus, Michel Morange concludes in his *A History of molecular Biology* (p. 116): "It was not Avery's experiment, nor even Hershey and Chase's, but the discovery of the double helix that convinced the biological community that genes were composed of DNA, and that it was thus the basis of heredity."

We may define scientific discoveries that are "either passively neglected or actively resisted at the time they are made" as *Premature*. To go back to Stent: "A discovery is premature if its implications cannot be connected by a series of simple logical steps to cannonical, or generally accepted knowledge." Hershey and Chase's paper came after Erwin Chargaff demolished the tetranucleotide hypothesis for DNA structure. Chargaff seems never to have overcome the oversight of his contribution to preparing the mind for the age of DNA.

An example of an unprepared mind that I find especially instructive is that told in 1961 by the psychologist Kerrich of a colleague of his in physics at the University of Witwatersrand:

> Prof. G. T. R. Evans used to demonstrate to his students an experiment where he was trying to pass an electric current through a chemical solution. The current would pass in the one direction, but not in the other. He noticed that one of the electrodes was dirty and polished it and then the current passed happily in either direction. Years later he read of an important new discovery: an oxide that permitted a current to pass in one direction but resisted passage in the other direction.

Prof. Evans realized that he scraped away in the rug the discovery of the transistor!

[The transistor is a product of research on the physics of solids, and particularly of those materials such as germanium and silicon known as semiconductors, the transistor was invented by John Bardeen, Walter H. Brattain, and William B. Shockley at Bell Telephone Laboratories in the US in 1947. It was discovered that crystals of semiconductors, which have the capacity to conduct electricity in some conditions and not in others, could be made to perform the function of a thermionic valve but in the form of a device that was much smaller, more reliable, and more versatile.]

But what are "postmature" discoveries? The term was introduced by Harriet Zuckerman (at the Department of Sociology in Columbia University) and Joshua Lederberg (of The Rockefeller University) in a short *Commentary* in *Nature* in 1986.

To the best of my knowledge, it has been overlooked ever since. Zuckerman & Lederberg suggest that postmature discoveries are "those which, are judged retrospectively to have been 'delayed'."

In 1946, as a young graduate student in Tatum's laboratory, Josh Lederberg performed a quite simple experiment, demonstrating "Gene recombination in *Escherichia coli.*" Thus providing evidence that sexuality, or at least processes analogous to sexuality, do exist in bacteria, and that bacteria may be amenable to genetic analysis, just as were *Drosophila*, mice, corn, or *Neurospora*. It was a postmature discovery. Its implications were connected by a series of already long–established "logical steps to cannonical, or generally accepted knowledge."

The notion of "postmature", like that of "premature" discoveries, is actually a comment on the *discontinuity* of scientific discoveries.

One question that may be asked is whether such a notion of discontinuity is helpful. I assume that most scholars who have considered the non–synchronization of scientific imagination, methodological and technological feasibility, and its socio–cultural atmosphere, would agree that such discontinuities are inevitable consequences in the history of science. However, the increasing number of papers around the question whether Mendel was a Mendelian may be conceived as a challenge to this notion of discontinuity.

Another question is whether a distinction between premature and postmature discoveries is meaningful, or at least helpful.

Zuckerman and Lederberg suggested that a postmatue discovery is one that "evokes surprise from the pertinent scientific community that it was not made earlier." In other words, it is the social relations and feelings of the scientists involved that make the difference. Contrary to the feeling of "what an ingenious ideas that was" attached to a premature discovery, the feeling of "how blind/stupid we were not to have seen it" makes the difference. Certainly, the distinction is to a large extent a question of the choice of a vantage–point. Although Mendel is said to have commented once "My day will come", from *his* vantage–point, deVries' "rediscovery" may be conceived as a postmature discovery. Similarly, from Griffith's point of view, and certainly from that of Avery, MacLeod and McCarty's, Hershey and Chase's and Watson & Crick's presentation were indeed postmature.

Zuckerman & Lederberg qualify that for a discovery to be judged in retrospect as postmature, it must have been

1. Technically achievable at an earlier time with the methods then available.
2. Understandable, capable of being expressed in terms comprehensible to working scientists at the time.
3. Its implications must have been capable of having been appreciated.

It seems that these criteria can differentiate premature from postmature discoveries *in retrospect*. In hindsight, Mendel's work should not be considered postmature because it failed criterion 2 (and probably also 3), Avery et al.'s discovery failed criterion 3. The discovery of recombination in bacteria was a postmature discovery because it fulfilled all three criteria: Mating and hybridization were the *sine qua non* conceptual as well as technical instruments of genetic research. The belief that bacteria lack sex–life was long overdue, certainly since prosperous sex life was described in different fungi (to which bacteria have been related in 1875 as *Schizomycetes* or 'fission fungi').

One could come up with a similar argument with respect to Levine's 'tetranucleotide' theory of DNA, it was long overdue. Nevertheless, even after Chargaff finally demolished it in 1950, scientists did not know what to do with it, or, "its implications were not capable of having been appreciated." As far as I can see it, what clinched the issue was Watson & Crick's notion that biological information is stored and conveyed essentially in a one–dimensional sequential manner, rather than in a three–dimensional structural manner.

> [It took still a couple of years to establish the notion that the 3–D structure of proteins too, is a function of the primary sequence of the amino–acids. An important regression to 3–D thinking was Monod & Changeux's "allosteric model"].

This allowed geneticists to appreciate the implications of Avery et al.'s discovery. Lederberg's discovery was 'postmature' because geneticists did know what to do with sex – actually they did not know what to do *without* sex. The introduction of sequential information rather than that of three dimensional one finally provided geneticists with a handle to build on DNA rather than protein. Avery et al., were premature.

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Are there other discoveries that may be classified as postmature ones? Discoveries that in the eyes of the scientific communities involved could have been made earlier?

I want to suggest two "discoveries" in the history of modern genetics that appear to me to qualify as postmature ones:

1. The notion of 'conditional lethals' and their utilization as an analytical tool.
2. The notion of the evolving evolutionary system and more specifically that of 'directed mutations'.

Some other cases that come to mind are

3. The discovery of different rules for the organization of the genetic material in eukaryotes than in prokaryotes, and more specifically the discovery of "redundant DNA" by Britten & Kohne in 1968.
4. The discovery of apoptosis, programmed cell–death in the 1990s.

I want to suggest that another characteristic of postmature discoveries is the explosion in the number of scientific papers that follow it within a short time. (Of course, this reflects exactly the subjective feeling that the discovery was long overdue, or in the air, for quite some time). The "band–wagon" effect may be a good indicator for "postmature" discoveries. Lederberg's discovery of recombination in bacteria qualifies. This is also the case with all four discoveries that I mentioned.

The term *Conditional lethals* was introduced in 1966 by Bob Edgar as a tool for the analysis of genetic control of the construction of the bacteriophage T4. Edgar thought that conditional lethals "might be a general method for finding mutations whose products were essential for phage development." (Allan M. Campbell, *Perspectives on Genetics*, p. 331). However neither the notion nor its application were new to geneticists. "Conditional" mutants were known since the early days of genetic research. Wolttereck developed the notion of the Norm of Reaction – the range of potential phenotypes that a genotype may develop if exposed to a specific range of environmental conditions – already in 1909. The effect of temperature on the hair color of Himalayan rabbits or of light on the seed color of 'sun–red' corn ears is described in the textbook *Principles of* Genetics of Sinnott and Dunn already in the 1932 edition. Lethal mutations were important components of genetic analysis since Muller's *ClB*–method for detection of mutations, in the 1920s. Lethality, conditioned

on Rh–incombatibility was known since the 1940s. The *K–pn* mutation, killing specifically *pn*–eyed *Drosophila* flied was described by Sturtevant in 1955. "Temperature mutants" were utilized by Horowitz and Leupold already in 1948 for detecting loss of indispensable functions of *Neurospora* (Horowitz & Leupold 1951, CSH 16, 65–74). And above all, Benzer's work on fine structure analysis of the *rII* cistrons of the T4 bacteripphage, raised the consciousness of the manner in which conditional lethals could be most constructively classified and analyzed. Indeed, Bob Edgar (1966) raised the question of why there was such a long time lag between the isolation of *ts* mutations in *Neurospora crassa* and the rediscovery of the principle with morphogeneisis of T4.

> [By (1) isolating mutant strains of phage T4 with temperature–sensitive and suppressor–sensitive conditional lethal mutations in each of the approx. 50 genes, and (2) analyzing the structures that accumulate when these mutant strains are grown under restrictive conditions by EM and biochemical techniques, R. S. Edgar, W. B. Wood, J. King, and colleagues were able to work out almost the entire pathway of phage T4 morphogenesis.]

Was it the elegance of the experimental work or the catching power of the term "conditional lethals" that made the high–resolution power of this mutational dissection of biological processes so popular as late as the middle of the 1960s? This is open for speculation. Anyhow, since then conditional lethals became a basic tool in the elucidation of the pathway of morphogenesis in organisms from bacteriophage, to bacteria to *Drosophila,* from yeast to Zebra–fish. (W. B. Wood, R. S. Edgar, J. King, I. Lielausis, & M. Henninger, 1968. *Federation Proceedings* 27, 1160–6).

> [The *kidney*–eyed mutant of the wasp *Habrobracon juglandis* (now called *Bracon hebetor*) was found in 1934 to be lethal at 30°C, but fully viable at lower temp (Whiting, P. W. 1934, *Genetics* 19, 268–291).
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> In *Drosophila melanogaster* it was especially David Suzuki et al. who have analyzed numerous conditional lethals and shown that the restrictive condition may often be localized to a specific "monophasic" period of development.

This year's Noble Prize, awarded to Lee Hartwell, was given to him for his utilizing conditional–lethal mutations for the analysis of the genes that control mitotic cell cycle in *Saccharomyces cerevisieae.*]

As early as 1939 C. D. Darlington published a book, *The Evolution of Genetic* Systems. A revised edition was published in 1958. In the chapter on 'Genotypic control' Darlington notes that "Frequency of mutation ? is controlled by the genotype" (p. 109). Evidence for genetic control of the mutational processes was not lacking. For example, Demerec described a specific mutator gene in Corn in 1937, and Mel Green described a series of such genes in Drosophila in 1973. In *E. coli* too, Treffers et al. described a mutator gene in 1954. And genetic systems that repair mutations or premutational events were thoroughly investigated for many years. Yet, it came as a shock to the community of geneticists when in 1988 John Cairns, Overbaugh and Miller came up with evidence that environmental conditions might be instrumental in causing directed mutations (Cairns, J., Overbaugh, J., & Miller, S. (1988). The origin of mutants. *Nature, 335*, 142–145). There was a general cry of disbelief. "There is a unicorn in my garden" complained Frank Stahl, and offered the sixty–four thousand $ prize to the one who would resolve the paradox. The paradox was, of course, that Cairns et al.'s experiment countered the notion of the pre–adaptive nature of bacterial mutations, as demonstrated in the classical experiments, primarily the 'fluctuation test" of Luria and Delbrück of 1943, but also those of Newcombe (1950) and Lederberg (1952), several years later. To quote one comment made a couple of years after the publication of Cairns et al.'s paper: "It is difficult to resist speculating that few people would have taken much interest in the field and few of the more provocative papers would have surfaced had it not been for (i) the thoroughly justified eminence of the senior author of the original paper, (ii) the acceptance and publication of the paper by *Nature*, and (iii) the crucial interpretation of Val–resistant control experiment. If there is indeed a strange animal in the garden, we must examine its diet very carefully before concluding that it is a unicorn" (Macphee, D. G. (1993). Directed mutation: paradigm postponed. *Mutation Research, 285*, 109–116).

It took some time for the genetic community to realize that this discovery was, actually, postmature. A year later, in 1989, when Lederberg discussed his and Cavalli–Sforza's 1956 studies on the isolation of preadaptive mutants in bacteria, he noted: "We must have an open mind about evolutionary specializations where metabolic alterations can target DNA itself. This might sometimes lead to ? adaptive genetic changes specifically induced by an environmental stress" (*Perspectives on Genetics,* p. 90). Soon the notion of directed mutations was integrated with the

notions of mutation–repair and it became acceptable that the mutagenic systems themselves were open to the evolutionary processes of mutations and Darwinian selection.

Of the three criteria for a postmature discovery, Technical achieveblity; understandablity; and appreciation of its implications, it was only the third one that was missing in the two examples given here. Once this was overcome, the notions had an increasing impact on genetic analysis.

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Already in 1967 Evellyn Witkin suggested that the messy polymerases found in bacterial cells may be mutagenic, and in November 1970, Miroslav Radman stunned his colleagues with the heretical proposal that bacteria harbor a genetic program to make mutations. Radman predicted them to be an element of the inducible SOS repair system, allowing individual cells to mutate in stressful situation, when their survival is threatened. In such situations increase in mostly deleterious genetic diversity could be a cost worthwhile paying for a chance to survive.

In the last five years it was found that several enzymes, which were considered to be helpers in the SOS repair system, are actually error–prone DNA–polymerases in their own right, "designed to generate mutations." Radman suggests calling these polymerases '*mutases*'. "Unlike the replicative DNA poymerases, which faithfully copy DNA sequences, mutases produce errors at high rates" (Radman, M. 1999. Enzymes of evolutionary change. *Nature, 401*(6756), 866–869).

Evidence suggests that these mutases are part of stress inducible processes, allowing them to function only when high mutation rates are advantageous. Whereas bacterial DNA pol V (and eukaryotic DNA pol ζ and η) can copy damaged DNA, allowing it to replicate and the cell to survive, DNA pol IV presumably acts on *undamaged* DNA, producing apparently 'gratuitous' mutations.

Thus Radman went one step further (Radman, M. 2001. Fidelity and infidelity. *Nature* 413(6852), 115): "[I]n real life it is survival, not fidelity, that is the ultimate virtue. Because adaptability involves exploration of genetic possibilities to fit ecological niches, molecular infidelity and repetition are more likely to succeed than a precise, non–repetitive process. ? A precise, single shot would often miss a target of uncertain position, whereas successive, imprecise firing will eventually lead to a hit." High efficiency must sacrifice high fidelity. "DNA replication is efficient and

therefore relatively imprecise." It would take too long to get DNA synthesizing right in the first place. The very precise process of DNA replication is achieved by leaving mistakes to error–correction enzymes. Radman suggests that this conflict of interest between fidelity and efficiency may have much wider implications in the evolution of living systems, allowing the evolution of 'error–prone' systems for special needs (such as the immune antibody–forming systems) or circumstances (such as stress situations) (**Fig.**). 'Proofreading' of DNA replication is only one function of this balance of forces and interests of living systems.

Do Radman's notions comprise a premature or a postmature discovery (or just another dead end)? We do not know. The decision can be made, if at all, only in retrospect, and then, it will depend on the context and the vantage–point, as does everything else in science (and in life).